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Anti-galectin-1 autoantibodies in human *Trypanosoma cruzi* infection: differential expression of this β-galactoside-binding protein in cardiac Chagas' disease

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SUMMARY

The pathogenesis of Chagas' disease has been subject of active research and still remains to be ascertained. Galectin-1 (Gal-1), a member of a conserved family of animal β-galactoside-binding proteins, localized in human heart tissue, has been suggested to play key roles in immunological and inflammatory processes. In the present study we demonstrated the occurrence of anti-Gal-1 autoAb in sera from patients in the acute and chronic stages of Chagas' disease (ACD and CCD) by means of ELISA and Western blot analysis. We found a marked increase in the level and frequency of Ig E anti-Gal-1 antibodies in sera from patients with ACD, but a low frequency of Ig M anti-Gal-1 immunoreactivity. Moreover, Ig G immunoreactivity to this β-galactoside-binding protein was found to be correlated with the severity of cardiac damage in CCD, but was absent in nonrelated cardiomyopathies. We could not detect immunoreactivity with *Trypanosoma cruzi* antigens using a polyclonal antibody raised to human Gal-1 and no hemagglutinating activity could be specifically eluted from a lactosyl-agarose matrix from parasite lysates. Moreover, despite sequence homology between Gal-1 and shed acute phase antigen (SAPA) of *T. cruzi*, anti-Gal-1 antibodies eluted from human sera failed to cross-react with SAPA. In an attempt to explore whether Gal-1 immunoreactivity was originated from endogenous human Gal-1, we finally investigated its expression levels in cardiac tissue (the main target of Chagas' disease). This protein was found to be markedly upregulated in cardiac tissue from patients with severe CCD, compared to cardiac tissue from normal individuals.

Keywords autoantibodies Chagas' disease galectin-1 heart *Trypanosoma cruzi*.

INTRODUCTION

Chagas' disease, which results from infection with the protozoan *Trypanosoma cruzi*, is endemic in many countries from Latin America where 16–18 million people may be infected. Acute Chagas' disease (ACD) is usually a mild illness and the severity of the pathology is clearly related to the intensity and the distribution of the parasite [1]. The microorganisms parasitize a variety of tissues, including the heart and symptomatic myocarditis develops in a small percentage of patients. Although cardiac pathology seems to predominate, the acute illness resolves spontaneously over a period of four to six weeks in most patients and the infection may then enter a quiescent stage with no apparente disease progression: the indeterminate phase of *T. cruzi* infection. Years, or even decades after *T. cruzi* infection is acquired, symptomatic chronic Chagas' disease (CCD) developes in about 30% of infected people. The heart is the target organ and CCD cardiopathy can be classified in the spectrum of dilated cardiomyopathies, which symptoms may include changes in electrical conductivity and apical left ventricular aneurysm [2–3].

The pathogenesis underlying the different forms of Chagas' disease has been subject of active research and still remains to be ascertained [4]. Nevertheless, autoimmunity is one of the mechanisms postulated to explain the development of pathological changes [4–8]. Furthermore, there is still scarce information about markers of progression of *T. cruzi* infection.

Galectin-1 (Gal-1) belongs to an evolutionary conserved family of animal β-galactoside-binding proteins, which exert their functions by cross-linking specific glycoconjugates [9–10]. Although the precise functions of individual members of this protein family have been difficult to assess *in vivo*, by virtue of their overlapping specificities [11], Gal-1 has been suggested to play key roles in immunological and inflammatory processes [12–14]. Its presence has been localized within the central and

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peripheral immune compartments in thymic epithelial cells [15], activated T cells [16], and inflammatory and activated macrophages [17,18]. It has been also found in immune privileged sites of the body such as placenta, cornea and testis [19–21]. More importantly, its expression has been reported in human cardiac tissue, the main target of Chagas' disease [22,23]. Interestingly, antigalectin autoantibodies have been found in sera from patients with inflammatory, autoimmune and neoplastic processes [24–26].

In the present study we demonstrated the occurrence of anti-Gal-1 autoantibodies in sera from patients with *T. cruzi* infection. We found a marked increase in the level and frequency of Ig E anti-Gal-1 antibodies in sera from patients with ACD, but a low frequency of IgM anti-Gal-1 immunoreactivity. Moreover, IgG autoantibodies reactive with this β-galactoside-binding lectin were detected in sera from patients with CCD and correlated with the severity of cardiac damage. Finally, evidence is also provided to show that Gal-1 is differentially expressed in heart tissue from patients with CCD, suggesting that this protozoan could increase the transcription of *gal-1* gene in human heart as a mechanism of immunomodulation.

PATIENTS AND METHODS

Reagents

Horseradish peroxidase-conjugated goat anti rabbit IgG, horseradish peroxidase-conjugated goat anti human IgG (γ-chain specific) and IgM (μ-chain specific), alkaline phosphatase-conjugated goat anti-human IgE (ε-chain specific) and electrophoresis reagents were purchased from Sigma Chemical Co. (St. Louis, MO). Human rGal-1 was produced as described [27] and kindly provided by Drs J. Hirabayashi and K.I. Kasai. The hemagglutinating activity was measured as previously described [18] and the NH_2-terminal amino acid sequence was determined with an ABI 477 A pulsed liquid sequencer (Applied Biosystems, Inc., Foster City, CA, USA). Lipopolysaccharide content of the purified sample was 60 ng/mg protein determined with a colorimetric endotoxin determination reagent (Pyrodick, Seikagaku, Tokyo, Japan). The rabbit polyclonal antihuman Gal-1 antibody was obtained as previously described [27]. The antibody was highly specific for Gal-1, since it did not recognize other members of the galectin family by Western blot analysis. All other chemical reagents were commercially available analitical grade.

Human sera

Twenty-one sera from patients with ACD (aged 6 months to 12 years old) were used in this study. These patients living in north-eastern Argentina, a well-known endemic area, presented inoculation chagoma, a portal-of-entry sign characterized by painless unilateral oedema of the eyelids. The patients showed parasitemia, detectable by at least one of the classical tests for parasite demonstration (microhaematocrit, Strout test or xeno-diagnostic). Samples were collected before the beginning of parasiticidal drug treatment. Thirty-eight sera from patients with CCD (average age: 40 years old) with positive serology for Chagas' disease (indirect hemagglutination, ELISA and immunofluorescence tests) were included. The later were divided into three disease severity groups: GI group ($n = 8$) showing no cardiac symptoms, normal electrocardiograms (ECG) and normal chest radiography (CXR) films; GII group ($n = 16$) corresponding to patients with normal CXR, but with ECG abnormalities and GIII patients ($n = 14$) showing abnormal ECG, signs and symptoms of congestive heart failure and cardiomegaly on CXR.

Control sera were obtained from 10 healthy individuals (average age: 45 years old) with negative serology, from the same endemic areas. Twelve sera from patients with nonchagasic cardiomyopathies were also analysed (average age: 54 years old). The cause of heart failure in these control patients was coronary artery disease or dilated cardiomyopathy. Procedures used in this study were approved by the Local Ethical Committee.

Human heart tissues

Adult human hearts were provided by *Hospital Privado* and *Hospital Misericordia* from Córdoba, Argentina. Control hearts ($n = 3$) were obtained 4 h *post mortem* from male patients who died in an accident. Chagasic hearts ($n = 3$) were obtained from patients with CCD undergoing cardiac transplantation.

Heart tissue extracts were prepared as previously described [28], with slight modifications. Briefly, tissues were homogenized in 10 volumes of ice-cold lysis buffer containing 50 mM Tris-HCl, pH 7·5, 150 mM NaCl, 1% NP-40, 10 mM EDTA, and a protease inhibitor cocktail (1 mM PMSF, 1 μg/ml leupeptin, 1 μg/ml pepstatin A, 10 μg/ml aprotinin and 10 mM iodoacetamide) and left on ice for 30 min. The solution was centrifuged at 4°C for 20 min at 10 000 × g and the supernatant was collected and stored at − 70°C.

Anti-human Gal-1 ELISA

Serum levels of antirGal-1 IgM, IgE and IgG were determined by ELISA. Briefly, microtitres plates (Nunc, Rochester, NY, USA) were coated with 5 μg/ml human rGal-1 in 50 μl phosphate-buffer saline (PBS), blocked and incubated with a 1 : 100 dilution of human sera. Bound IgM and IgG were detected by incubation with horseradish peroxidase-conjugated goat anti human IgM (1 : 600) and anti human IgG (1 : 600), followed by substrate (orthofenilendiamine). Bound IgE was detected using a 1 : 5000 dilution of an alkaline-phosphatase-conjugated goat anti human IgE, followed by substrate (dinitrophenyl phosphate). All secondary antibodies were purchased from Sigma Chemical Co (St. Louis, MO). Plates were washed three times between steps with 0·01% Tween-20/PBS (v/v). Optical densities were measured at 490 or 405 nm, respectively, in an ELISA reader (BioRad, Richmond, CA). A serially diluted polyclonal rabbit antihuman rGal-1 revealed with a peroxidase-conjugated antirabbit IgG was used as a control of positive reactivity.

Western blot analysis

SDS-PAGE was performed in a Mini Protean-II electrophoresis apparatus (Bio Rad) as described [18]. In order to assess the presence of anti-Gal-1 antibodies in patients sera, recombinant Gal-1 (5 μg) was resolved on a 15% separating polyacrylamide slab gel. After electrophoresis, the separated protein was transferred onto nitrocellulose membranes and probed with appropriate dilutions of human sera from ACD and CCD patients from the different groups. Blots were finally incubated with a peroxidase-conjugated antihuman IgG or IgM (1 : 300).

In another set of experiments, extracts corresponding to heart tissues derived from patients with CCD or from victims of accidents, were resolved on a 15% polyacrylamide slab gel and transferred onto nitrocellulose membranes. Blots were incubated with a 1 : 500 dilution of the rabbit antihuman Gal-1 antibody followed by a peroxidase-conjugated goat antirabbit IgG.

Reactions were developed using 4-chloro-1-naphthol (Sigma Chemical Co.). Control of specific immunoreaction was performed by incubation of the blots with a rabbit preimmune serum. Equal protein loading of normal and CCD heart tissues was checked by Coomassie blue staning, showing the total protein profile. Moreover, Ponceau S red staning gave also evidence of equal loading after transference to nitrocellulose membranes.

Finally, *T. cruzi* epimastigotes were lysed using 1% NP-40 and protease inhibitors as described above, and the presence of a Gal-1-like structure was analysed by Western blot on a 15% polyacrylamide slab gel using the polyclonal anti-Gal-1 antibody.

Affinity chromatography of T. cruzi epimastigotes

To attempt the purification of Gal-1 from *T. cruzi* epimastigotes, parasites (~5 g) were washed twice with PBS and sequentially homogenized with PBS containing 4 mM 2-ME, 2 mM EDTA and protease inhibitors (MEPBS) supplemented with 100 mM lactose. Parasites were disrupted by five cycles of sonication at 12 kHz for 5 s at 4°C, and the total cell lysate was centrifuged at 600 × *g* for 10 min to remove cell debris. The whole cell lysate was then applied to a lactosyl-agarose matrix (Sigma Chemical Co.) as described [18–20]. Briefly, lysed parasites were dialysed against MEPBS to remove lactose and applied at a flow rate of 5 ml/h to the lactosyl-agarose matrix previously equilibrated with MEPBS. The column was thoroughly washed with MEPBS until no absorbance at 280 nm was detected in the effluent. Finally, the adsorbed material was specifically eluted with MEPBS containing 100 mM lactose and collected in 1-ml fractions. Then, after extensive dyalisis against MEPBS, fractions were monitored for protein content and hemagglutinating activity using trypsin-treated glutaraldehyde-fixed rabbit erythrocytes. All procedures were conducted at 4°C unless stated otherwise.

Immunoadsorption

In order to examine potential crossreactivity between Gal-1 and SAPA, immunoadsorption assays were performed as previously described [29]. Briefly, Gal-1 (5 μg/ml) was adsorbed on nitrocellulose membranes. After adequate washing and blocking, the immunosorbents were incubated overnight at 4°C with 1 ml of 1 : 20 dilution of ACD or control sera. Bound antibodies were eluted with 0·02 M glycine, pH 2·8, neutralized with 1 M Tris, pH 8·6. Each eluted sample was tested against SAPA (5 μg/ml) and rGal-1 (5 μg/ml) by ELISA using conjugated anti human IgM and anti human IgE antibodies.

Statistical analysis

The Mann–Whitney *U*-test was used to compare nonparametric data for statistical significance using the Instat computer package. The Fisher′ exact test was used to compare the percentage of positive *versus* negative sera for each antibody isotype.

RESULTS

Detection of anti-Gal-1 IgM and IgE antibodies in acute Chagas' disease.

Anti-galectin autoantibodies have been identified in sera from patients with inflammatory and autoimmune diseases [24–26]. Since the ethiopathogenic mechanisms of *T. cruzi* infection involve inflammatory and autoimmune processes, we challenged the question whether anti-Gal-1 antibodies could occur in Chagas' disease.

A quantitative determination of anti-Gal-1 antibodies was performed by ELISA in sera from ACD and CCD patients (classified as GI, GII and GIII according to the severity of cardiac damage). A variable level of anti-Gal-1 antibodies corresponding to the IgM isotype were mainly detected in ACD patients (Fig. 1a). As clearly shown, this reactivity was observed in sera corresponding to ACD patients in 12 out of 21 samples, compared to control sera ($P = 0{\cdot}005$). This was confirmed by Western blot

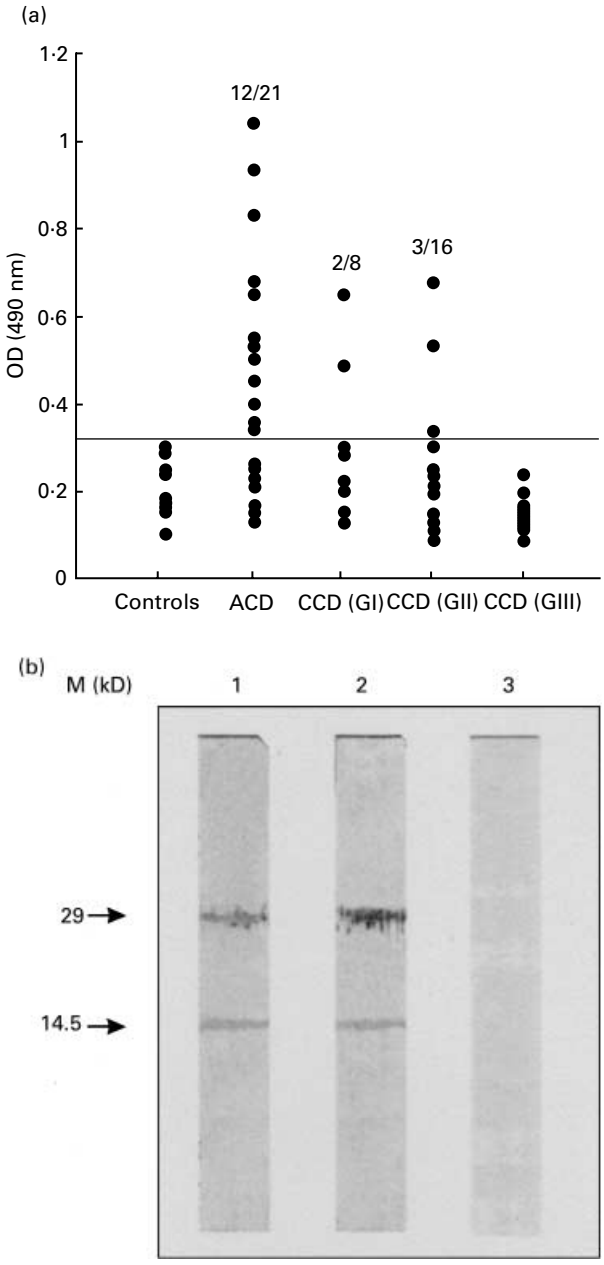

Fig. 1. IgM reactivity against rGal-1 in sera from controls, ACD and different groups of CCD. (a) ELISA: each symbol represents the mean value of duplicates, obtained with each serum assayed at a 1 : 100 dilution. The horizontal line indicates the mean + 2 SD, obtained with control sera. $*P = 0{\cdot}005$ (ACD *versus* controls). (b) Western blot: IgM immunoreactive profile of sera from ACD patients (1 : 200 dilution) against rGal-1 (lanes 1 and 2). Sera from control individuals were processed in parallel (lane 3). Molecular weight markers are indicated on the left.

analysis, showing the 14·5 kD monomeric band and the dimeric functionally active 29 kD band (Fig. 1b). On the other hand, CCD patients did not exhibit any significant IgM reactivity against rGal-1 (P = NS).

Next, we investigated the occurrence of anti-Gal-1 IgE antibodies in sera from patients with ACD and CCD. As shown in Fig. 2, a highly significant IgE reactivity was detected by ELISA in 19 out of 21 samples from ACD patients compared to CCD patients and controls ($P < 0{\cdot}0001$). Interestingly, both the frequency of anti Gal-1 IgE antibodies in the acute phase of the disease and the high level of reactivity, suggest that these antibodies could be potential markers of ACD. To test the possibility that IgE antibodies are recognized by rGal-1 through lectin sugar interactions [30], thiodigalactoside or lactose were incubated with serum samples before the ELISA reaction. Galectin-specific sugars were not able to displace the binding of IgE to rGal-1 (data not shown), suggesting that the carbohydrate recognition domain (CRD) of the galectin is not implicated in the interaction with IgE.

Reactivity of anti-Gal-1 IgG antibodies in CCD patients with severe cardiac damage

In search for anti-Gal-1 reactivity in sera from patients with progressive Chagas' disease, we found that the greatest IgG reactivity was tightly associated with the most severe cardiac damage. As shown in Fig. 3a, IgG reactivity by ELISA was found in 6 out of 14 CCD patients from GIII with severe cardiomiopathy, in comparison with CCD patients from GII and GI stages. This result was highlighted by the strong reactivity against rGal-1 detected by Western blot analysis (Fig. 3b). As expected, immunoreactivity, determined by the intensity of the bands, increased according to the severity of the disease in stages GI, GII and GIII (lanes 4, 5 and 6, respectively).

An overview of the percentages of positive sera, analysing the

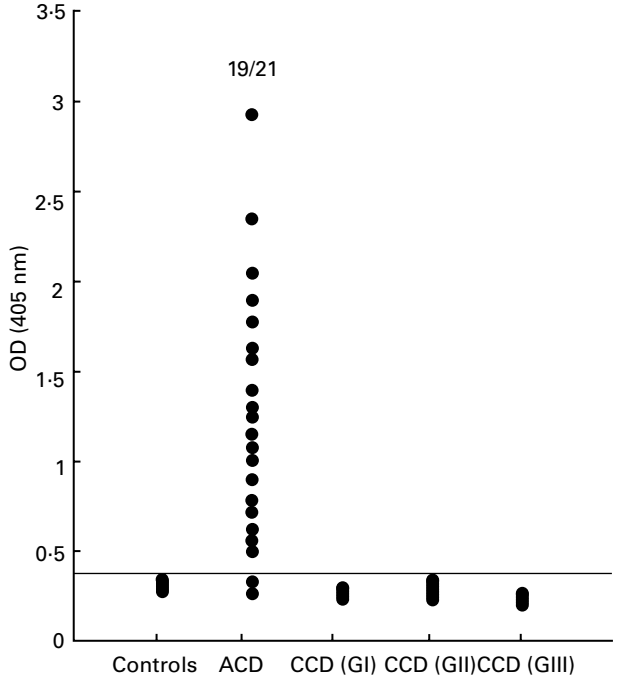

Fig. 2. IgE reactivity against rGal-1 in sera from controls, ACD and different groups of CCD patients. ELISA: each symbol represents the mean value of duplicates, obtained with each serum assayed at a 1 : 100 dilution. The horizontal line indicates the mean + 2 SD, obtained with control sera. *$P < 0{\cdot}0001$ (ACD *versus* controls).

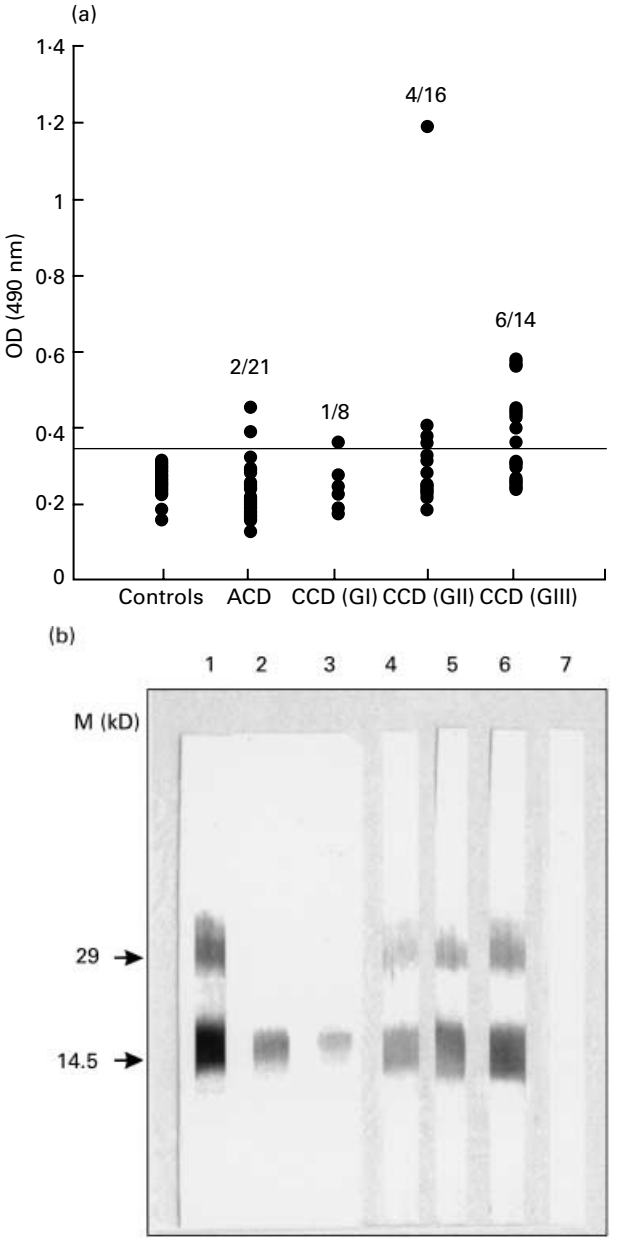

Fig. 3. IgG reactivity against rGal-1 in sera from controls, ACD and different groups of CCD patients. (a) ELISA: each symbol represents the mean value of duplicates, obtained with each serum assayed at a 1 : 100 dilution. The horizontal line indicates the mean + 2 SD, obtained with control sera. *$P = 0{\cdot}05$ (CCD GIII *versus* controls); *P = N.S. (ACD *versus* controls). (b) Western blot**:** IgG immunoreactive profile of sera from CCD patients (GI, GII and GIII) against rGal-1 (lanes 4, 5 and 6, respectively). Recombinant Gal-1 was resolved on a 15% polyacrylamide slab gel, transferred to nitrocellulose membranes and tested with a 1 : 100 dilution of each serum. Sera from control individuals were assayed at the same dilution (lane 7). The immunoreactive protein bands are indicated by the arrows. Positive controls were assayed by blotting rGal-1 (5, 1 and 0·5 μg) with the rabbit antihuman Gal-1 antibody (lanes 1, 2 and 3, respectively).

different isotypes in the different stages of the disease is summarized in Fig. 4. The highest frequency of reactivity in ACD patients corresponded to the IgE isotype (90·5%) ($P < 0{\cdot}0001$), followed by IgM isotype (57·1%) ($P = 0{\cdot}004$) and the lowest frequency was found to be associated with the IgG isotype (9·5%). No reactivity was found for the IgE isotype in sera from CCD patients at any of the evolutive stages of the disease. Moreover, an intermediate frequency of IgM and IgG reactivity was observed in sera from patients corresponding to GI and GII stages. Finally, sera from CCD patients belonging to the GIII stage, who were affected by the most severe cardiomiopathy, exhibited the highest frequency of positive IgG reactivity against this 14·5 kD β-galactoside-binding protein (42·8%) ($P = 0{\cdot}024$). In order to determine that the reactivity against rGal-1 was specific of cardiac damage in Chagas' disease, we tested whether sera from patients with other unrelated cardiomyopathies (coronary artery disease or dilated cardiomyopathy) exhibited antirGal-1 immunoreactivity. We could not detect neither IgG nor IgM reactivities against rGal-1 in any of the samples assayed (Table 1).

Galectin-1 is not present in T. cruzi; investigation of potential cross-reactivity between Gal-1 and other parasite antigens

In order to investigate whether anti-Gal-1 antibodies were originated from an immunogenic Gal-1 structure present in the parasite, we attempted different experimental strategies using cultured *T. cruzi* epimastigotes. We could not detect any significant immunoreactivity using the specific polyclonal anti-Gal-1 by Western blot analysis of epimastigotes extracts or by immunofluorescence staining of the whole parasite. Moreover, no hemagglutinating activity could be detected when whole parasite

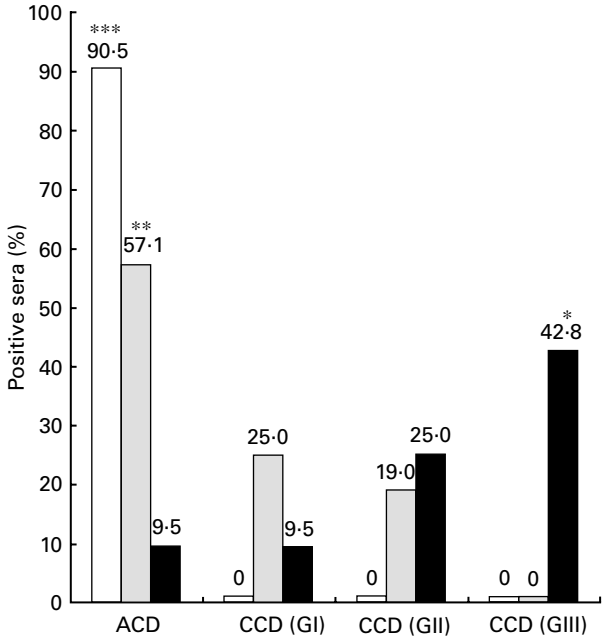

Fig. 4. Percentage of sera from ACD and different groups of CCD patients reactive with rGal-1. Analysis of IgE (□), IgM (▨) and IgG (■) isotypes.***$P < 0{\cdot}0001$ (IgE isotype ACD *versus* controls); **$P = 0{\cdot}004$ (IgM isotype ACD *versus* controls); *$P = 0{\cdot}024$ (IgG isotype CCD *versus* controls).

Table 1. IgM and IgG antibody reactivity against rGal-1 in sera from patients with non-chagasic cardiomyopathy (NCC) by ELISA

Patient group	Absorbance at 490 nm (mean ± SD)	
	IgM anti-rGal-1	IgG anti-rGal-1
NCC	0.163 ± 0.067	0.128 ± 0.035
Control	0.263 ± 0.096	0.137 ± 0.032

lysates were applied and further eluted from a lactosyl-agarose matrix (data not shown).

In search for potential cross-reactivity between Gal-1 and parasite antigens, using the SWISS PROT data base, we found a significant sequence homology between Gal-1 and the lectin domain of shed acute phase antigen (SAPA). The Gal-1 sequences corresponding to the residues 44–70 and 50–62 showed high homology with the SAPA sequences 829–855 (37·0%) and 815–827 (46·2%), respectively. This antigen, which has been shown to be highly immunogenic during natural and experimental infection, belongs to the *trans*-sialidase family from *T. cruzi* and displays β-galactoside binding activity [31]. It has been shown to elicit an antibody specific response in 95% of patients in the acute phase of the disease [32]. Hence, we investigated whether anti-Gal-1 specific antibodies in ACD could arise from cross-reaction with SAPA. For this purpose, Gal-1 (5 μg/ml) was fixed to nitrocellulose filters and incubated with sera from three ACD patients. Bound antibodies were further eluted from nitrocellulose as previously described [29], and exposed to SAPA in an ELISA assay. Immunoreactivity was visualized after incubation of ELISA assays using a peroxidase-conjugated antihuman IgM antibody or an alkaline phosphatase conjugated antihuman IgE antibody. Despite considerable sequence homology between these two antigens, we could not find any cross-reactivity when eluted antibodies from patients sera were further exposed to SAPA-coated wells in an ELISA assay and revealed using an anti-IgM antibody (Fig. 5a). Moreover, similar results were observed under the same experimental conditions using the anti human IgE antibody (data not shown). As expected, high immunoreactivity was observed when sera from ACD patients were exposed to SAPA (Fig. 5a). As a positive control we also tested whether immunoadsorbed anti-Gal-1 antibodies could still bind human Gal-1 in an ELISA assay when eluted from nitrocellulose membranes. We detected a strong reactivity of eluted antibodies against rGal-1 (Fig. 5b). Furthermore, the rabbit anti human Gal-1 polyclonal antibody failed to recognize SAPA (data not shown). These results indicate that antibodies against Gal-1 which are present in human sera do not cross-react with SAPA.

Galectin-1 expression is up-regulated in cardiac tissue from patients with CCD

Since Gal-1 has been isolated from human cardiac tissue [22,23] and heart is the most commonly affected organ in Chagas' disease, we finally investigated whether Gal-1 could be differentially expressed in heart tissue from CCD patients with severe cardiac damage, in comparison to normal tissues. For this purpose, extracts were prepared and processed for protein detection by Western blot analysis, using the rabbit anti human rGal-1 antibody as described [14]. As clearly shown in Fig. 6a, Gal-1 expression

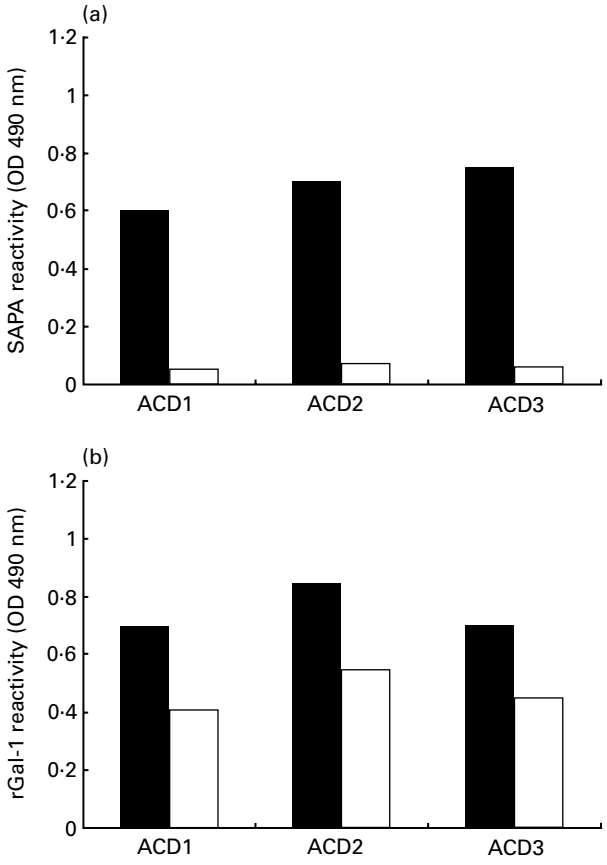

Fig. 5. Anti-Gal-1 antibodies eluted from ACD patients sera do not cross-react with SAPA. Sera from three patients (■) or the eluted anti-Gal-1 antibodies (□) purified by immunoadsorption were tested against (a) SAPA and (b) rGal-1 by ELISA. Immunoreactivities were detected using a peroxidase-conjugated antihuman specific IgM antibody. Similar results were obtained using an alkaline phosphatase-conjugated anti human IgE antibody (data not shown).

was found to be up-regulated both in its monomeric (14·5 kD) and dimeric (29 kD) forms in heart tissue extracts from CCD patients (lane 3, 50 μg) in comparison with control tissue extracts (lane 1, 50 μg). This differential expression was found to be particularly evident when lower concentrations from heart tissue extracts (10 μg) were analysed by Western blot (lane 4 *versus* lane 2). To check equal loading of proteins in each lane, the total protein profile of heart tissue extracts (50 and 10 μg) from control (lanes 1 and 2) and chagasic (lanes 3 and 4) samples is shown by Coomassie blue staining (Fig. 6b). This result suggests a potential relationship between endogenous Gal-1 expression in human heart and the generation of anti-Gal-1 IgG antibodies in patients with Chagas' disease affected by a severe cardiomyopathy.

DISCUSSION

In the present study we demonstrated a highly significant increase in the level and frequency of anti-Gal-1 IgE antibodies in sera from patients with ACD. Furthermore, we have identified the occurrence of anti Gal-1 IgG antibodies in sera from patients with CCD. This finding was tightly correlated with the severity of cardiac damage in these patients. Interestingly, we could not find any immunoreactivity in sera from patients with other unrelated cardiomyopathies.

Several hypothesis could be postulated to explain the occurrence of these antibodies in *T. cruzi* infection. First, *T. cruzi* could express an immunogenic Gal-1-like structure and trigger a humoral immune response against this evolutionarily conserved protein. Identification of homologous carbohydrate-binding proteins in different species ranging from fungi to mammals, dates the existence of common ancestors for the galectin gene family to more than 800 million years ago, suggesting that these endogenous lectins must be serving important biological roles [33,34]. In support for this hypothesis, several carbohydrate-binding proteins with specificity for mannose and galactose have been reported in the parasite [35]. We have explored this possibility by Western blot analysis and immunofluorescence staining of parasite epimastigotes using the specific anti-Gal-1 antibody and further purification through affinity chromatography on a lactosyl-agarose column. We could not identify the presence of Gal-1 or a galectin-related structure in this cycle stage of the parasite. Other possibility is that a Gal-1 related structure could be present in other life-stages of the parasite, such as trypomastigotes or amastigotes. In this regard, protein sequencing analysis revealed that human Gal-1 and the lectin-like domain of shed acute-phase antigen (SAPA) from *T. cruzi* share significant sequence homology. This antigen, which has been shown to be highly immunogenic during natural and experimental infection, belongs to the *trans*-sialidase family from *T. cruzi* and displays β-galactoside binding activity [31]. Interestingly, Affranchino *et al*. [32] reported the presence of antibodies reactive with SAPA in 93% of sera from patients at the acute phase of the infection, but in only 10% of patients at the chronic phase of the disease. Results presented in this study indicate the absence of cross-reactivity between Gal-1 and SAPA.

Since different glycoforms of IgE have been found to differentially bind Gal-3 (previously called ε-binding protein) [30], we examined the possibility that IgE from ACD patients could recognize the 14·5 kD Gal-1, through lectin–carbohydrate interactions. It has been reported that Gal-3 recognized sialidase-treated human IgE to a much greater extent than the untreated IgE [9]. In this regard, an attractive hypothesis could be that the trans-sialidase of *T. cruzi* may cleave sialic acid residues from IgE. As a result of desialylation, β-galactoside residues would be highly exposed, thus leading to an enhanced reactivity with Gal-1. To test this possibility, ELISA reactions were performed in rGal-1-coated plates by incubating serum samples in the presence of thiodigalactoside or lactose. Galectin-specific sugars were not able to displace the binding of IgE to rGal-1, suggesting that the carbohydrate recognition domain (CRD) of the galectin, was not implicated in this process.

Another suggestive explanation is that the parasite could infect host tissues including the heart and promote the release of endogenous Gal-1, which could lead to autoantibody production in the acute phase of infection. The occurence of high levels of IgE autoantibodies in autoimmune disorders has been previously reported [36,37].

Since the major cardiomiopathy is characteristic of CCD and the level of anti-Gal-1 IgG antibodies was associated with the severity of cardiac damage, we also investigated whether Gal-1 expression was differentially regulated in heart tissue, the main target of Chagas' disease. Experimental evidence is also provided in this study, showing that

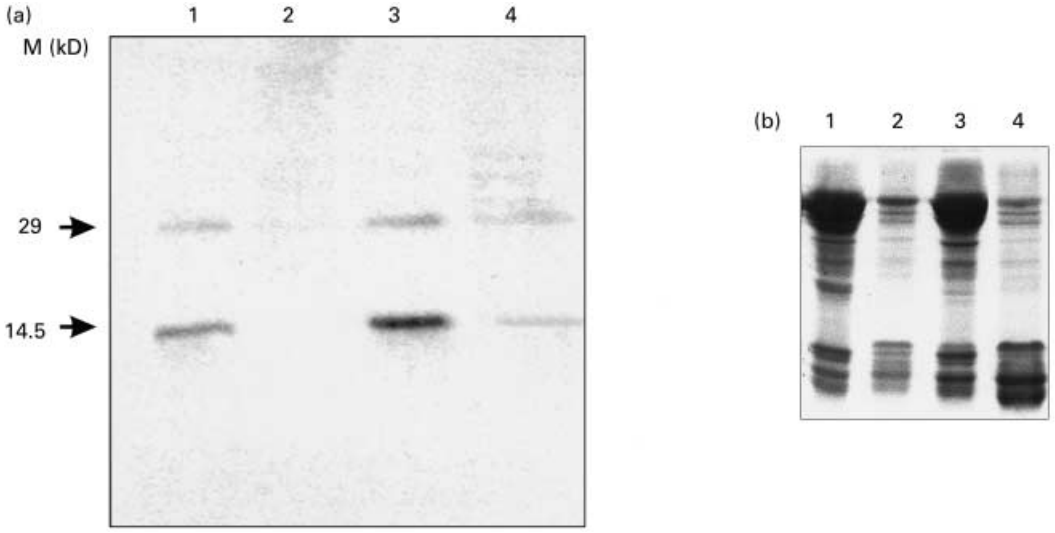

Fig. 6. Galectin-1 is differentially expressed in heart tissue from patients with CCD. (a) Heart tissue extracts corresponding to patients with CCD (lanes 3 and 4: 50 and 10 μg, respectively) and to control patients (lanes 1 and 2: 50 and 10 μg, respectively) were prepared and analysed by SDS-PAGE on a 15% polyacrylamide slab gel. Then, proteins were transferred onto nitrocellulose membranes and immunoblotted with a rabbit antihuman rGal-1 polyclonal antibody (1 : 500). The immunoreactive monomeric and dimeric forms of Gal-1 are indicated by the arrows. Results are representative of three independent experiments. (b) Gels were also stained by Coomassie blue to show the equal protein loading in comparable lanes.

expression of this β-galactoside-binding protein is upregulated in cardiac tissue from patients with CCD, compared to cardiac tissue from control individuals.

Galectin-1 immunoreactivity in patients sera may be a part of a more general enhanced autoimmune response to cardiac tissue in ACD and CCD patients. In this regard, evidence has been provided in patients and experimental models of Chagas' disease of an autoimmune response to heart components such as myosin, actin, and myoglobin [4,29,38].

Galectins have emerged as a new class of bioactive molecules with specific immunomodulatory properties [10]. Gal-1, a prototype member of this family has been involved in cell apoptosis [18,39–42], inflammation [14,43] and cell adhesion [44,45]. This protein family has been proposed to exert its functions by cross-linking specific oligosaccharide ligands on cell surface glycoconjugates. Accordingly, it has been shown that adhesion of *T. cruzi* to heart muscle cells is mediated by protein–carbohydrate interactions [46,47]. Hence, overexpression of Gal-1 on heart tissue could facilitate parasite invasion to this vulnerable site during the infection. Since Gal-1 has been shown to induce activated T-cell apoptosis [14,18,41], one might also speculate that the increased expression of Gal-1 in human cardiomyocytes, could mediate apoptosis as an homeostatic mechanism to shut off autoreactive T cells, which are highly activated during cardiac disease [8].

Investigation of the role of this β-galactoside-binding protein in parasite–host interactions and elucidation of the molecular nature of these anti-Gal-1 antibodies, will certainly contribute to in more depth understanding of the role of this carbohydrate-binding protein in immunopathology.

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